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Resistant starch from processed cereals: the influence of amylopectin and non-carbohydrate constituents in its formation

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Abstract

The effect of removal of protein and lipids on the resistant starch (RS) content of processed rice and ragi has been studied. The percent recovery of RS increased significantly (over 2–3-fold) after defatting and autoclaving. However, deproteinization did not show any major difference. Prolonged storage of gelatinized starch suspensions showed considerably more undigested mass even after extended α-amylolysis, probably due to the involvement of long unbranched chains of amylopectin in the formation of RS. Thermal characteristics of these processed flours/starches showed variable enthalpy values. X-ray diffraction data revealed both B- and V-type diffraction patterns for the isolated RS.

1. Introduction

Starch is the principal (dietary) energy-producing polysaccharide of plants. In the form of discrete granules, starch occurs in nature in varied shapes and sizes and differing physico-chemical and functional characteristics (Tharanathan, Muralikrishna, Salimath, and Raghavendra Rao, 1987; Tharanathan, 1995). During food processing the dietary starch partly undergoes modification to an enzyme-resistant form, which is highly refractory for digestion by the gastrointestinal tract enzymes (Englyst, Wiggins, and Cummings, 1982). This undigested fraction of starch is called resistant starch (RS). Its formation depends on several factors such as pH, temperature of heating and cooling, moisture content, etc. Recently, the qualitative and quantitative make up of RS from processed rice and ragi was reported (Mangala, Malleshi, Mahadevamma, and Tharanathan, 1997). In this communication we will report on the effect of the various non-carbohydrate constituents such as protein and lipids, and also the involvement of amylopectin in the formation of RS.

2. Materials and methods

Certified varieties of rice (*Oryzae sativa*, intan) and ragi (finger millet, *Eleusine coracana*, indaf), procured from local suppliers, were cleaned, sun-dried, and ground in a plate mill. Only reagent-grade chemicals were used. Protease (Type-XXIII- *Aspergillus oryzae*, E.C. No. 3.2.2.4), pancreatic α-amylase (E.C. No. 3.2.1.1), amyloglucosidase (E.C. No. 3.2.1.3), peroxidase (E.C. No. 1.11.1.7) and glucose oxidase (E.C. No. 1.1.3.4) were from Sigma Chemical Co., USA, and Termamyl was from Novo, Denmark.

Starch was isolated from the processed cereal flours by the water steeping method (Madhusudhan and Tharanathan, 1995).

Hot paste viscosity of flours and starches was determined by the Rapid visco analyser model 3D fitted with a thermo-regulator set to 6°C. The slurry (3 g in 26 ml H_20) was finally heated to 95°C for 5 min before allowing it to cool to 50°C. Viscosity in RVU was recorded throughout the process of starch gelatinization (heating) and retrogradation (cooling).

For deproteinization (Paramahans, Wankhede, and Tharanathan, 1980), the crude starch was suspended in aqueous medium at pH 9.0 (by adding dil. NaOH) for ~15 min with gentle stirring. The suspension was centrifuged and excess alkali was removed by repeated water washings. It was further deproteinized by stirring with 0.1 M NaCl–toluene (10:1, v/v), for ~2 h, thrice, and later centrifuged, excess NaCl was removed by repeated water washings and finally the starch was dried by the solvent exchange method.

Defatting of surface lipids in native or deproteinized starch was done by treatment with solvents such as

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methanol-petroleum ether (1:3, ME), 1,4-dioxan (D), 70% aqueous *n*-propanol (70-P) and water-saturated *n*-butanol (WSB) at refluxing temperature for 2 h (× 2). They were filtered over a Buchner funnel and dried as above. Defatting of total lipids was done by prior acid hydrolysis (8N HCl, ~95°C, 30 min), followed by extraction with petroleum ether. The extracted fat was then dried to constant weight. Determinations were performed in duplicate.

2.1. RS isolation

Defatted and/or deproteinized starches were autoclaved (starch:water, 1:3, w/v) at 125°C, 15 psi pressure for 1 h and cooled to room temperature (1 h) and at 4°C (12 h). This was repeated four times, and finally the samples were treated with a α-amylase (Termamyl, 0.05 ml) at boiling water bath temperature for 45 min, followed by a protease (10 mg ml^{-1} of phosphate buffer, 0.08 M, pH 7.5) at 37°C. After centrifugation, the residues were rehydrolysed with amyloglucosidase (6 mg in 1 ml acetate buffer, 0.05 M, pH 4.6 at 60°C) for 35 min. The undigested mass (crude RS), separated by centrifugation, was dissolved in dil. KOH solution (2M), neutralised (dil. HCl) and again subjected to amylolysis with amyloglucosidase (Mangala et al., 1997). The released glucose was measured by the glucose oxidase method (Dahlqvist, 1964).

2.2. Retrogradation studies

Starch samples (250 mg in 3 ml water) were autoclaved at 125°C for 1 h in the presence of calcium propionate (1.7 mg) and allowed to retrograde for 48 h at room temperature (Type A-RS), 24 h at 6°C followed by 48 h at 40°C (Type B-RS) and 24 h at 6°C followed by 20 days at 40°C (Type C-RS). The retrograded samples (250 mg) were then incubated with a mixture of pancreatic α-amylase (15 mg) and amyloglucosidase (15 mg) in sodium acetate buffer (0.1 M, pH 5.2 at 37°C) for different intervals of time (1, 2, 4, and 6 h) and the released glucose was assayed.

2.3. X-ray diffraction

X-ray diffraction patterns were determined using an EG-7G solid state germanium liquid N_2 cooled detector Scintag XDS-2000 instrument equipped with a θ–θ goniometer at 25 mA and 30 kV. The starch samples were powdered to pass through a 150 mesh sieve and kept for saturation with distilled water in a desiccator overnight. The samples were exposed for 5 h to Co-K_α filtered radiation (λ 1.54184 nm) (Zobel, 1964). Diffractograms were scanned from 2 to 400 at a diffraction angle of 2θ.

2.4. Differential scanning calorimetry (DSC)

This was performed with a DSC-Rheometric Scientific (UK) instrument equipped with thermal software ver 5.40. The samples (5–10 mg) at a moisture content of 58–80% were heated from ambient to 100°C at a programmed rate of 5°C min^{-1} (for defatted and native starch/flour) and 10°C min^{-1} (for retrograded starch and RS); indium was the reference standard used (Russell & Juliano, 1983).

3. Results and discussion

Native rice and ragi starches have a total lipid (surface and internally bound) content of 0.7 and 1.3%, respectively. Out of the four different solvents tried, WSB and 70-P extracted relatively more (>80%) of surface lipids from the starch granules. Defatting was most efficient (over 86% fat removal) with 70-P followed by WSB, D and ME, in agreement with a report on wheat starch (Eerlingen, Van Haesendonck, DePaepe, and Delcour, 1994c). Being more hydrophilic, it is likely that 70-P exerts a better defatting power, due to preswelling and partial gelatinisation of starch taking place during refluxing. It has been shown that solvents containing too little or no water result in less swelling of starch and a lower yield of RS (Morrison, 1981). It has been shown that 70-P has a defatting efficiency of 99% on wheat starch, and the resulting defatted material was almost completely gelatinised with almost a total loss of starch birefringence characteristics (Eerlingen et al., 1994c), whereas defatting with ME caused only minor changes.

As a result of defatting, considerable granule swelling was observed even at low pasting temperatures. Compared to native starch values, all the defatted starches showed lower viscosity, especially so in ragi samples (Table 1). In part this may be due to the presence of any residual solvent (especially alcohols) in the defatted material. It is known that low molecular weight compounds such as alcohols, lipids, etc. can bind ~6 times their own weight of amylose; for example, 0.54% lipids can theoretically bind ~3.24% of amylose (Morrison, 1988; Karkalas and Raphaelides, 1986). However, deproteinized starches did not show a viscosity lowering effect.

In comparison with native starch granules, which showed an A-type X-ray diffraction pattern Fig. 1(a) and Fig. 2(a), the defatted samples showed diffractogram characteristics of V-type starches Fig. l(c) and Fig. 2(c). This has been attributed in part to the gelatinization of starch induced during defatting treatment with hot aqueous solvents, which also brings about partial loss of granule crystallinity and birefringence (Kugimiya, Donovan, and Wong, 1980). Amylose-lipid

complexes are also shown to possess V-type diffraction patterns (Eerlingen, Cillen, and Delcour, 1994a). On the other hand, deproteinized starches showed 2θ peaks having A-type diffraction pattern. Deproteinized rice starch showed peaks at 15.4, 15.7, 17.5, 18.4 and 23.8 Fig. 1(b) whereas deproteinized ragi starch showed the same at 15.5, 17.4, 18.3 and 23.5 Fig. 2(b).

From the DSC results (Table 2), it is evident that the endothermic transition temperature of defatted starch considerably decreases in comparison to undefatted native starches. Removal of lipids (and protein) is known to affect starch gelatinization characteristics (Morrison, 1981). This is probably because the defatted

Table 1
Pasting viscosity indices of defatted and deproteinized starches

Sample	GT (°C)	Viscosity (RVU)			
		PV	HPV	CPV	SBV
Rice starch, native	77.4	160	97	225	128
Deproteinized	76.0	161	85	154	69
Defatted					
ME	73.0	64	62	99	37
D	67.3	140	105	192	87
WSB	73.5	111	110	174	63
70-P	76.2	22	20	35	13
Ragi starch, native	72.9	148	94	163	69
Deproteinized	73.7	146	81	180	99
Defatted					
ME	72.5	139	112	275	163
D	70.6	130	131	232	101
WSB	71.7	151	112	275	163
70-P	77.7	26	45	45	14

GT, gelatinization temperature; PV, peak viscosity; HPV, hot paste viscosity; CPV, cold paste viscosity; SBV, set back viscosity (CPV−HPV).

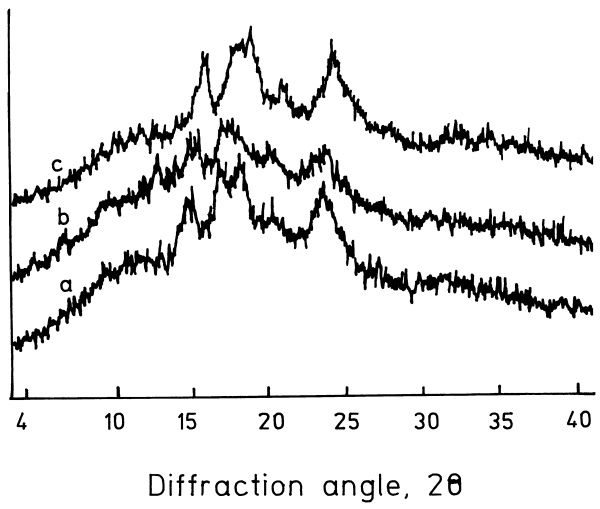

Fig. 1. X-ray diffractograms of rice starches: (a) native; (b) defatted with 70-P; (c) deproteinized.

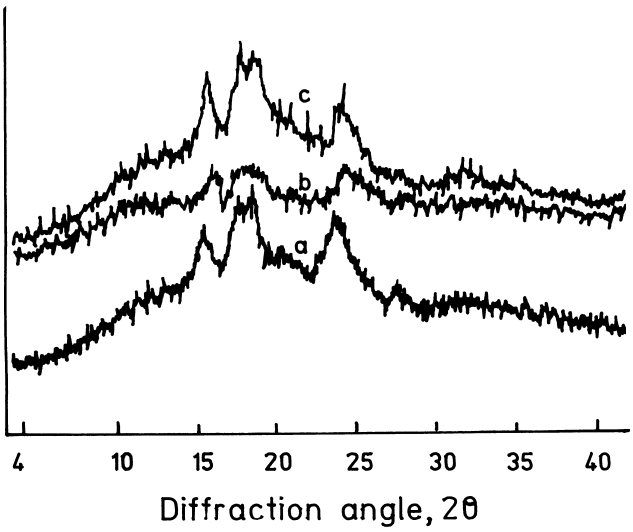

Fig. 2. X-ray diffractograms of ragi starches: (a) native; (b) defatted with 70-P; (c) deproteinized.

Table 2
DSC characteristics of defatted starches

Sample	Endothermic transition temperature (°C)				
	To	Tp	Tc	ΔT (Tc−To)	ΔH (mcal mg^{-1})
Rice starch, native	59.1	69.6	83.4	24.3	5.60
Rice starch, defatted					
ME	46.6	63.9	78.3	31.7	3.39
D	48.6	60.9	72.2	23.6	5.33
WSB	53.6	59.6	85.4	31.8	2.26
70-P	43.6	54.6	64.8	21.2	1.82
Ragi starch, native	59.6	65.3	72.3	12.7	9.80
Ragi starch defatted					
ME	45.8	62.9	73.4	27.6	9.26
D	60.4	66.3	77.9	17.5	7.94
WSB	58.7	75.0	84.6	25.9	7.50
70-P	49.1	54.0	64.4	15.3	1.12

To, Tp and Tc are onset, peak and conclusion temperatures; ΔT, temperature range of gelatinization; ΔH, enthalpy.

Table 3
Recovery (%) of RS in native, defatted and deproteinized starches

Sample	Before autoclaving		After autoclaving	
	Rice	Ragi	Rice	Ragi
Native	0.02	0.009	0.03	0.01
Deproteinized	0.14	0.04	0.15	0.10
Defatted				
ME	0.14	0.05	0.39	0.15
D	0.16	0.06	0.38	0.15
WSB	0.18	0.06	0.70	0.12
70-P	0.22	0.07	0.89	0.13

sample can hold considerably more water, as reported previously (Lorenz and Johnson, 1972). The decrease in enthalpy (ΔH) was to a greater extent in 70-P defatted samples for reasons unknown. Nevertheless, the findings are in good agreement with earlier literature data (Eerlingen et al., 1994a, c).

Table 4
DSC characteristics of retrograded rice and ragi starches

Sample	Endothermic transition temperature (°C)				
	To	Tp	Tc	ΔT (Tc−To)	ΔH (mcal mg^{-1})
Rice starch					
Type-A	44.8	57.8	64.2	19.4	0.60
Type-B	50.9	58.3	68.8	17.9	1.12
Type-C	54.8	59.4	72.6	17.8	1.24
Ragi starch					
Type-A	62.9	66.6	71.3	8.4	0.73
Type-B	63.8	67.5	72.0	8.2	1.21
Type-C	65.2	69.0	73.0	7.8	1.32

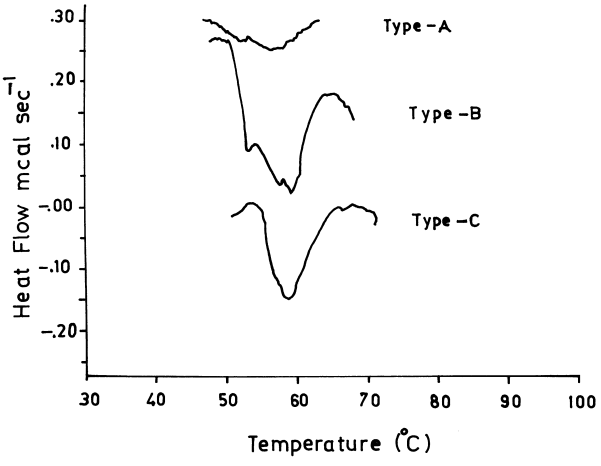

Fig. 3. DSC thermograms of retrograded rice starches.

Table 3 shows the yield of RS from native and defatted rice and ragi starches. RS yield increased significantly after defatting and autoclaving, especially with the 70-P defatted material. It is understandable that, as a result of defatting, fewer lipid molecules, especially internally bound lipids, are present for complexing with amylose and therefore, due to the availability of more free, uncomplexed linear fraction, after repeated heating and cooling treatments it undergoes leaching and an extensive aggregation, resulting in an entangled 3D-network of RS (Eerlingen et al., 1994a). In support of this, it has been shown that addition of exogenous lipids to autoclaved high amylomaize barley starch reduces the percent yield of RS, due to amylose-lipid complexing, as indicated by both DSC and X-ray diffraction data (Eerlingen, Jacobs, and Delcour, 1994b). The presence of lipids not only has an impact on RS yield, but also influences RS quality. Both lipids and some sugars, such as glucose, maltose, sucrose and ribose, have been shown to have a significant influence on the overall RS yield from processed food materials (Eerlingen, Van Den Broeck, Delcour, Slade, and Levine, 1994d). However, deproteinization had no effect on RS content.

To study the involvement, if any, of the amylopectin component of starch in the RS formation, the autoclaved starch samples were left for different intervals of time. In contrast to what is observed with amylose, crystallization of amylopectin is an extremely slow process, continuing over a period of several days. Nevertheless, the long extended A and B chains (particularly those of an unbranched nature) of amylopectin may still undergo hydrogen bonding to a certain extent (Levine and Slade, 1990) and indirectly this may contribute to the net yield of RS. In tune with this, the stored retrograded samples were not fully digested with amylase even after 6 h amylolysis, in comparison with the freshly gelatinized samples which were digested in less than 3 h. In DSC, the melting endotherm of amylopectin was seen at 60°C whereas that of amylose was around 150°C

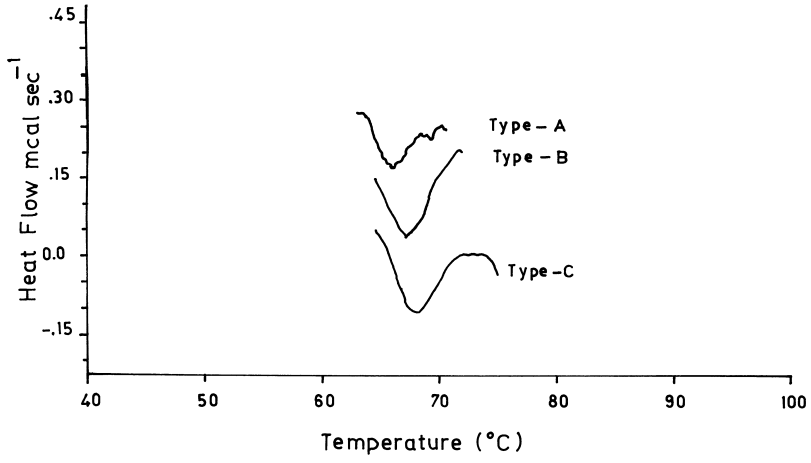

Fig. 4. DSC thermograms of retrograded ragi starches.

(Eerlingen et al., 1994b). The X-ray diffractograms of retrograded rice and ragi starches showed major peaks at 13.2, 15.2, 17.2, 17.6, 20.2 and 20.8, which corroborated well with the reported literature values for B-type or V-type starch (Levine and Slade, 1990). Peaks in ragi retrograded starch were not that conspicuous, probably because of its low moisture level. Longer storage of autoclaved-cooked quinoa samples results in retrograded amylopectin crystallites, which have a bearing on its RS content (Ruales and Nair, 1991). Amylopectin retrogradation, the first physico-chemical change in bread crumb, was found to be the major cause of crumb firming.

DSC analyses of Type-A, Type-B and Type-C retrograded starches and their endothermic transitions are given in Table 4 and Figs. 3 and 4. The values for ragi samples were slightly higher than those of rice. Nevertheless, a significant increase in the To, Tp and Tc and also in ΔH, over that of native starch was observed for the Type-C sample. A better crystal perfection probably occurs for Type-C retrograded starch, because of its extended time of storage at 40°C.

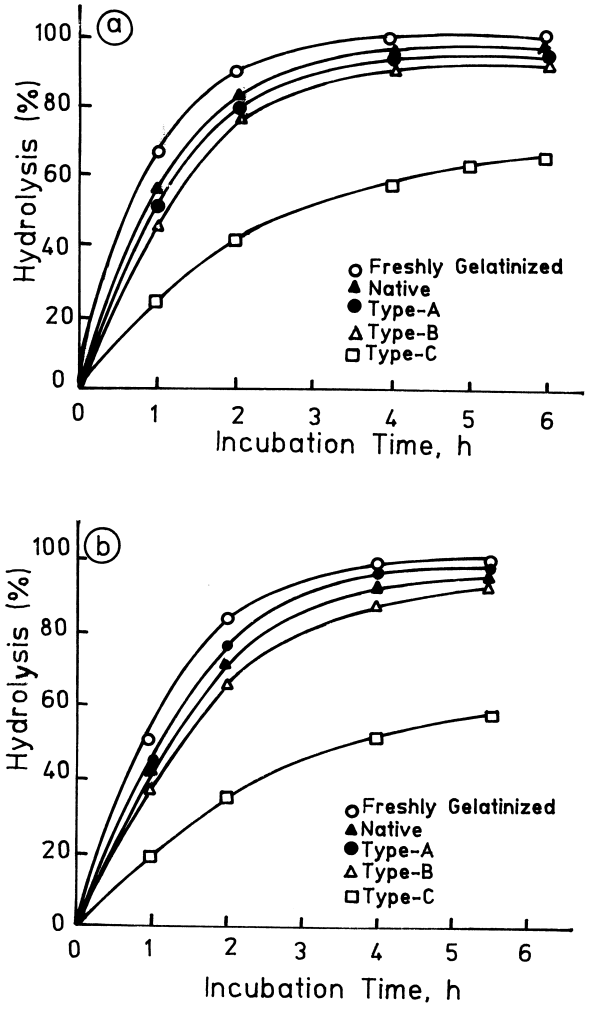

Fig. 5. Retrogradation characteristics of (a) rice and (b) ragi starches.

The in vitro amylolysis data (Fig. 5) showed that freshly gelatinized samples hydrolysed very rapidly (in < 3 h), indicating the absence of a definite crystalline network, whereas the Type-C samples were relatively more resistant for amylolysis, probably because of a higher degree of entanglement, and therefore a higher crystallinity index. Even after 6 h of incubation, Type-C starches showed only 60% hydrolysis in the case of rice and ~50% in the case of the ragi sample.

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